A COMPARISON OF THE RESPONSES OF TRITURUS AND DESMOGNATHUS TO THYROID-STIMULATING HORMONE ADMINISTRATION [1]

W. GARDNER LYNN [2] AND JAMES NORMAN DENT [3]

Biology Division, Oak Ridge National Laboratory,[4] Oak Ridge, Tennessee, Department of Biology, Catholic University of America, Washington 17, D. C., and University of Virginia, Charlottesville, Virginia

We have recently reported experiments comparing the effects of goitrogen treatment on the histology and the concentration of radioiodine by the thyroid glands of *Triturus viridescens* and *Desmognathus fuscus* (Dent and Lynn, 1958). The results indicate that, although these drugs markedly inhibit thyroidal concentration of iodine in both urodeles, characteristic histological changes in the gland occur in *Desmognathus* only. It was suggested that the failure of the *Triturus* thyroid to give the usual histological response to such treatment might be accounted for by either (1) some defect in the pituitary control of thyroid function, such that decreased thyroid hormone level in the blood does not cause any marked increase in TSH (thyroid-stimulating hormone) production, or (2) a lack of ability of the *Triturus* thyroid to respond to TSH stimulation. The present experiments were undertaken to test the latter alternative by study of the effects of administration of exogenous TSH upon histological structure and on radioiodine uptake in the thyroids of these two animals.

Materials and Methods

Specimens of *Triturus* (*Diemyctylus*) *viridescens viridescens* (Rafinesque) were collected from a pond near Monterey, Virginia. Specimens of *Desmognathus fuscus fuscus* (Rafinesque) were taken from a stream near Oliver Springs, Tennessee. The animals were kept in the laboratory for a minimum period of one month before being used for experiments. They were fed every other day, *Triturus* with ground beef fortified with cod liver oil and calcium phosphate, *Desmognathus* with live meal-worm larvae. Both before and during the experimental period the animals were kept in a constant temperature chamber at 23.0° ± 1.0° C.

Injections of 0.1 ml. of a solution of Armour's Thytropar (1.0 U.S.P. Unit per ml.) in 0.7% saline were made into the body cavities of the experimental animals on alternate days. The salamanders were always anesthetized in an aqueous solution of tricaine methane sulfonate (1 part in 1000) before injection.

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[2] Department of Biology, The Catholic University of America.

[3] Department of Biology, University of Virginia.

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For comparative study of the effects of injected TSH on the thyroids of *Triturus* and *Desmognathus*, groups of each species were given various numbers of injections and were killed various periods of time after the cessation of injection. The thyroid regions of these animals were fixed in equal parts of Bouin's fluid and ethyleneglycolmonoethyl ether. After sectioning they were stained with Harris' hematoxylin and Ponceau de Xylidine-Orange II (Gray, 1952).

Radioiodine uptake by and release from the thyroid was followed by a standardized procedure. Sixteen mature specimens of *Desmognathus* (2.8–3.3 g. body weight) and sixteen mature specimens of *Triturus* (2.5–3.3 g. body weight) were selected and divided into groups of eight controls and eight experimental animals. All animals were given six successive injections, experimental animals receiving the TSH preparation and controls receiving 0.1 ml. of 0.7% NaCl solution. Three hours after the last of these injections, each animal was given an additional intraperitoneal injection of 0.1 ml. of 0.7% saline containing 50.0 μc./ml. of I^{131}. At

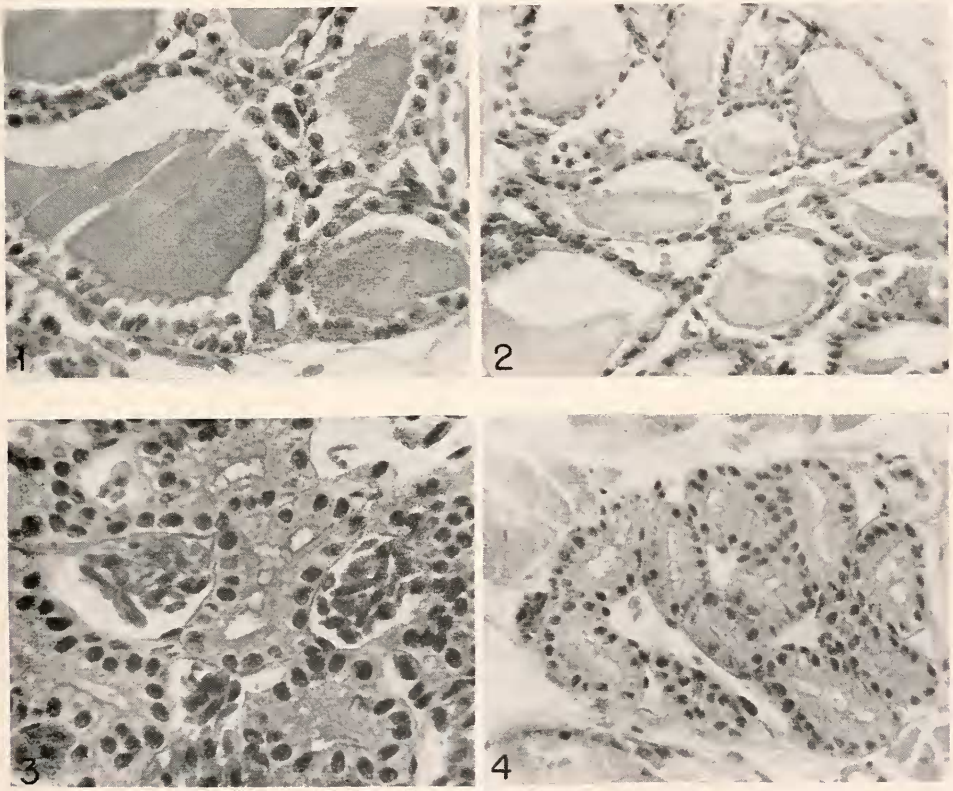

FIGURE 1. Section of thyroid gland of control specimen of *Triturus*.

FIGURE 2. Section of thyroid gland of control specimen of *Desmognathus*.

FIGURE 3. Section of thyroid gland of *Triturus* given seven successive injections of TSH on alternate days and killed 24 hours after the last injection.

FIGURE 4. Section of thyroid gland of *Desmognathus* given seven successive injections of TSH on alternate days and killed 24 hours after the last injection.

intervals of 6, 12, 24, 36, 48, and 60 hours thereafter,[5] each specimen was again anesthetized and the radioactivity of the thyroid and heart regions was measured by means of a previously developed technique (Dent and Lynn, 1958). In this technique a scintillation counter was used, consisting of a 1.5-inch NaI crystal cemented to the window of an RCA type 5819 photomultiplier tube and a conventional amplifier and binary scaler. The crystal and photomultiplier were mounted in a lead cylinder 5.2 cm. thick with a collimating slit measuring 4.0 by 12.0 mm. At the conclusion of the measurements of radioactivity, the animals were killed and their thyroids fixed and sectioned.

TABLE I

Radioactivity (corrected for physical decay) at the indicated periods of time after injection with 5 μc. of I^{131}. Experimental animals had previously received six successive injections of TSH. All means are based on observations on eight individuals.

Standard deviation: $s = \sqrt{\sum_{i}^{n} \frac{(X_i - \bar{X})^2}{n - 1}}$ *where* $n = 8$.

Species	Time after I^{131} injection (hours)	Radioactivity (cts./sec.)			
		Controls		Treated	
		Thyroid	Heart	Thyroid	Heart
Triturus viridescens	6	4.43 ± 1.686	3.92 ± 1.074	5.71 ± 1.479	4.08 ± 0.856
	12	5.03 ± 1.201	3.01 ± 0.558	7.01 ± 2.224	2.74 ± 0.525
	24	4.88 ± 1.179	1.33 ± 0.694	9.41 ± 4.057	1.44 ± 0.659
	36	5.07 ± 0.962	1.16 ± 0.352	9.39 ± 3.807	1.20 ± 0.727
	48	4.93 ± 1.022	0.91 ± 0.259	9.97 ± 4.916	0.86 ± 0.493
	60	4.96 ± 1.105	0.70 ± 0.315	9.09 ± 3.805	0.90 ± 0.699
Desmognathus fuscus	6	4.29 ± 1.040	6.14 ± 1.724	7.65 ± 1.492	5.11 ± 1.726
	12	5.35 ± 1.004	4.83 ± 0.812	6.16 ± 1.731	3.66 ± 1.327
	24	4.56 ± 0.765	3.24 ± 0.702	4.44 ± 1.452	2.57 ± 0.581
	36	3.75 ± 0.645	2.10 ± 0.579	3.97 ± 1.391	1.83 ± 0.580
	48	3.26 ± 0.569	1.62 ± 0.212	3.60 ± 1.930	1.68 ± 0.311
	60	3.02 ± 0.977	1.45 ± 0.215	3.16 ± 1.431	1.42 ± 0.403

RESULTS

1. *Effects of TSH treatment on thyroid histology*

It has previously been pointed out (Dent and Lynn, 1958) that, during the summer months, the thyroids of untreated *Triturus* present a quite uniform histological appearance characteristic of relatively low activity. The follicles are well rounded, the epithelium is low, the colloid is homogeneous and acidophilic, chromophobe droplets are absent or sparse (Fig. 1). The thyroids of untreated *Des-*

[5] In preliminary experiments, radioactivity measurements were made at hourly intervals for the first 8 hours after injection of radioiodine. It was found that the activity in the thyroid reached peak levels at 5–6 hours in *Desmognathus* but was still rising steadily in *Triturus* at 8 hours. In all later experiments the measurements were begun at 6 hours and continued for 60 hours at the intervals indicated.

mognathus, on the other hand, show more individual variation but all present an appearance of moderate to relatively high activity: cuboidal to columnar epithelium; less homogeneous, often basophilic, colloid; relatively abundant chromophobe droplets at the peripheries of the colloid masses (Fig. 2).

Administration of TSH caused marked changes in the thyroid in both. Thyroids of animals given seven injections (over a 13-day period) and fixed 24 hours after the last injection showed the maximal effects seen in this experiment and have been chosen for illustration (Figs. 3 and 4). It will be noted that, in both *Triturus* and *Desmognathus,* the epithelium increased greatly in height, the colloid was almost entirely discharged from the gland, and the follicles became irregular in shape. Study of the thyroids of animals given smaller numbers of injections

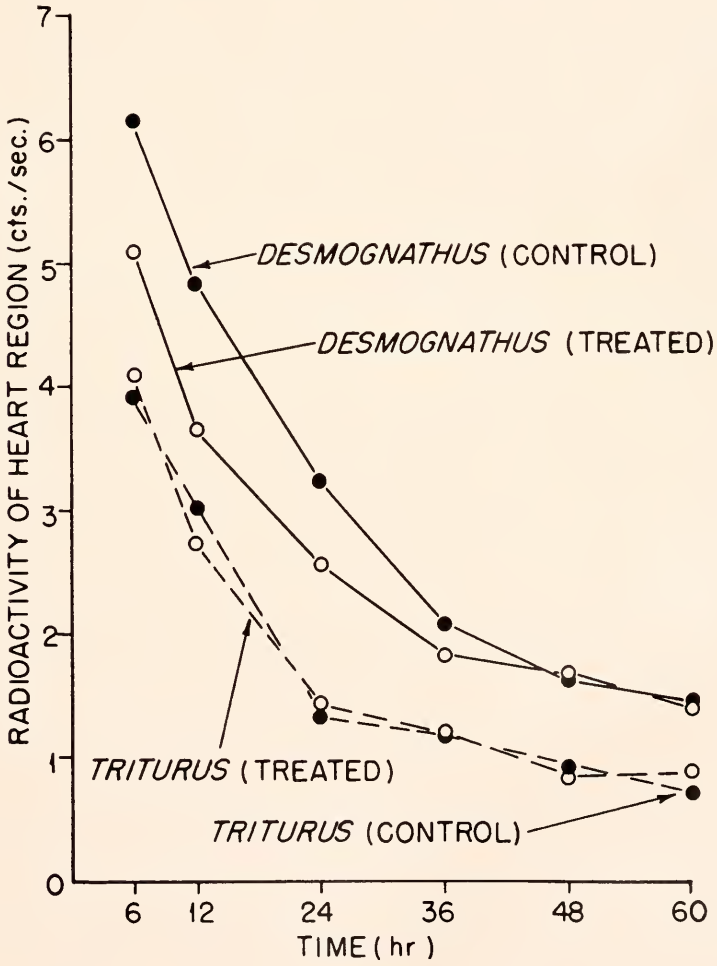

FIGURE 5. Radioactivity in the heart regions of experimental and control specimens of *Desmognathus* and *Triturus* over a period of 60 hours after administration of radioiodine.

shows that these same effects are obtained with six, five, or four injections. Thyroids of salamanders given three or two injections showed less marked but definite histological responses. Thyroids of animals that received a single injection could not be distinguished from those of controls.

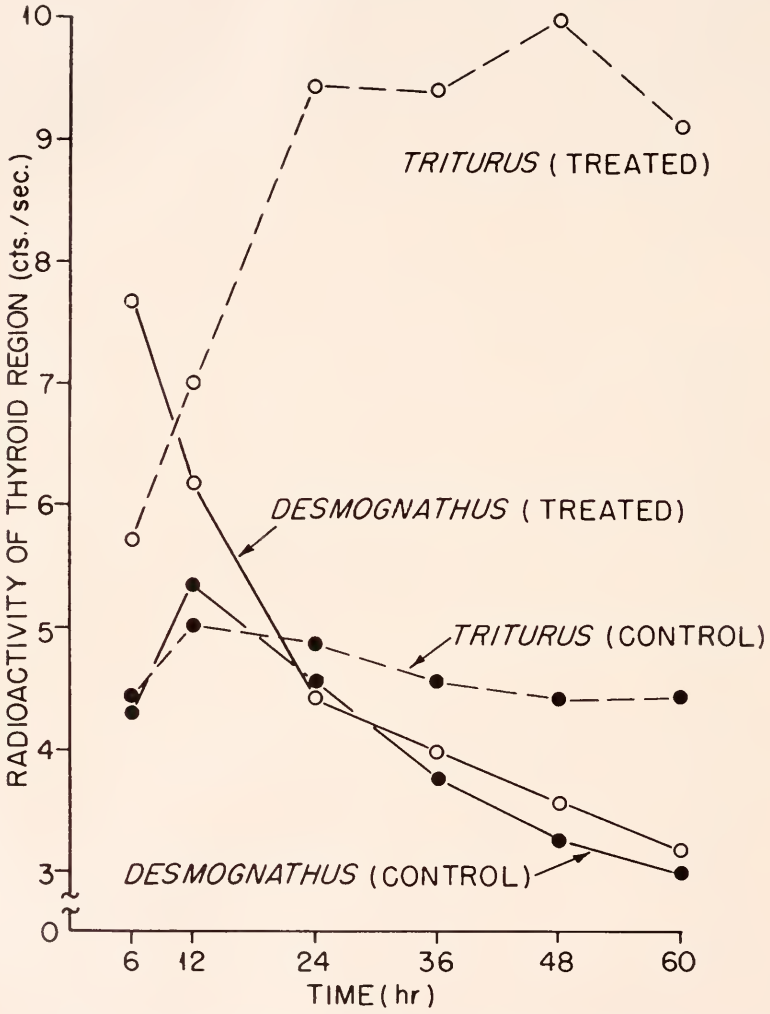

FIGURE 6. Radioactivity in the thyroid regions of experimental and control specimens of *Desmognathus* and *Triturus* over a period of 60 hours after administration of radioiodine.

By giving a sufficient number of injections of TSH to ensure marked responses (4–8 injections), and varying the times of fixation after the last injection, it was ascertained that the maximal histological response persists for 36–48 hours. Thyroids of specimens fixed 60 hours after the last TSH administration showed definite indication of regression toward the normal histology with a decrease in

epithelial height and the beginning of accumulation of stored colloid. By 75 hours regression was well under way and by 144 hours the thyroids of treated salamanders were like those of controls.

2. Effects of TSH treatment on uptake of radioiodine by the thyroid

The means of counts of radioactivity (corrected for physical decay) in thyroid and heart regions of control and experimental animals at successive periods after injection of I^{131} are shown in Table I, and these data are illustrated graphically in Figures 5 and 6.

It will be noted (Fig. 5) that the heart region, which we consider as representative of soft tissues other than those of the thyroid, shows relatively high counts at 6 hours after I^{131} administration in both control and experimental groups of both species. The counts then decline rapidly as the radioiodine is eliminated from the circulating blood. There is no significant difference between experimental and control groups within the species, but the counts for the *Triturus* heart region are uniformly lower than those for *Desmognathus.*

Counts for the thyroid region (Fig. 6) reveal that treatment with TSH results in an increased uptake of radioiodine by the gland in both *Triturus* and *Desmognathus.* At 6 hours after I^{131} injection, the counts in the thyroid regions of both groups of experimental animals are well above those in the corresponding controls. However, the later counts show important differences between the two species. In *Desmognathus,* the counts decline quickly and in 24 hours have reached the level of the control thyroids. Thus, the initial rate of uptake of radioiodine is high but the rate of turnover is also high and there is no significant retention of I^{131} in the thyroids of experimental animals as compared with those of controls after 12 hours. In *Triturus,* on the other hand, the counts rise more slowly in the period up to 24 hours after I^{131} injection and then level off with little sign of decline by the end of the experiment (60 hours). In this species, then, the TSH treatment caused increase in radioiodine uptake and this, combined with a slow rate of turnover, resulted in retention of I^{131} at a much higher level than in the controls. It may be noted that the curve for the thyroids of *Triturus* control specimens shows little change in counts throughout the counting period. This indicates the relatively low rate of activity of the thyroid in normal animals of *Triturus* which we have reported previously (Dent and Lynn, 1958).

Discussion

Our earlier experiments indicated that, although both *Triturus* and *Desmognathus* show a marked inhibition of thyroid function after treatment with goitrogenic drugs, the usual histological changes that occur after goitrogen treatment are not seen in *Triturus* during treatment periods of less than two months duration.[6] These histological changes are normally induced by an increased production of pituitary thyrotropin after a decrease in the level of circulating thyroid hormone. The absence of such changes in the *Triturus* thyroid could indicate either a failure of the pituitary to produce significantly higher levels of thyrotropin after thyroid

[6] Adams, 1946a, and Dent (unpublished) have shown that the thyroid of *Triturus* undergoes hyperplasia after several months of treatment with goitrogens.

inhibition or a failure of the thyroid to respond in the typical way to thyrotropin stimulation.

The results reported here show clearly that the *Triturus* thyroid is capable of responding to exogenous thyrotropin. The histological response of the *Triturus* thyroid proved to be entirely comparable to that of the *Desmognathus* thyroid and the effect of TSH treatment on radioiodine uptake was even more marked in the former than in the latter. These findings thus indicate that the histological signs of low thyroid activity in the normal gland of *Triturus*, and its failure to show signs of increased activity after goitrogen treatment, are to be attributed to a low level of thyrotropin production.

The fact that treatment with exogenous TSH causes such a marked increase in radioiodine uptake in *Triturus* as compared with *Desmognathus* may be taken as further evidence that TSH production is unusually low in *Triturus*. Several previous workers have concluded that the responsiveness of the thyroid to exogenous TSH varies inversely with the thyrotropin content or production of the animal's own pituitary (Loeb and Friedman, 1930; Collip and Anderson, 1935). Indeed, the selection of the young guinea pig and the chick as favorable animals for assay of thyrotropin is based on the relatively low thyrotropin content of the pituitary in these animals and the accompanying high sensitivity of their thyroids (Adams, 1946b). Among cold-blooded vertebrates the goldfish (*Carassius auratus*) is the best known example of a species in which the thyroid normally presents a histological appearance of very low activity. The goldfish thyroid, like that of *Triturus*, shows no histological evidence of increased activity after goitrogen administration (Fortune, 1955) but responds strongly to injected TSH (Gorbman, 1940; Berg and Gorbman, 1954). Fortune (1956) has suggested that in the goldfish the inactive thyroid, resulting from absence or very low level of TSH production, may be an important factor in this animal's exceptional ability to withstand high temperature. We have previously pointed out (Dent and Lynn, 1958) that this hypothesis may be valid for *Triturus* as well, since this salamander also has a high upper limit of temperature tolerance.

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Summary

1. Specimens of *Triturus viridescens* and *Desmognathus fuscus* were given injections of Armour's Thytropar on alternate days.

2. Histological examination of the thyroid glands of experimental and control animals showed that both species gave maximal response after only four injections, the follicular epithelium being greatly heightened and intrafollicular colloid almost completely discharged, and that the response persisted for 3–4 days after cessation of treatment, but then declined and disappeared by 7 days.

3. Measurements of uptake and turnover of I^{131} by the thyroids of animals given six injections of thyrotropin showed a high uptake and a rapid turnover in *Desmognathus*. In *Triturus* the effect of the treatment upon I^{131} uptake was also

marked and the radioiodine was retained at a high level throughout the counting period (60 hours).

4. It is concluded that the *Triturus* thyroid is more responsive to exogenous TSH than is that of *Desmognathus*, and that this is related to an exceptionally low TSH content and production in the anterior pituitary of *Triturus*.

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